Typification and distribution of two hybrids of *Juniperus communis* L.

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ABSTRACT

The common juniper (*Juniperus communis* L.) is a widely distributed species in the northern hemisphere, with numerous infraspecific taxa proposed over time. Eight groups are currently recognized, three of them occurring in Europe and the Mediterranean; they are considered as species, subspecies or varieties, with the country of origin of the taxonomists having a strong influence on this decision. Introgressions and hybrids have been recognized among some of these infraspecific taxa. This paper typifies two known hybrids between Eurasian varieties of *J. communis*, and discusses several confusing names proposed in this group, related with these nothovarieties. *Juniperus communis* nothovar. *intermedia* is a hybrid between *J. communis* var. *communis* and *J. communis* var. *saxatilis*, widely distributed in Europe, but sometimes ignored or confused with other taxa; several names applied to infraspecific taxa of this group actually seem to refer to this hybrid. It was described in 1851, but there is no known type; a neotype is proposed in this paper. A hybrid between *J. communis* var. *hemisphaerica* and *J. communis* var. *saxatilis* was reported in 2020, but not properly described; it is done in this paper. *Published online* ***www.phytologia.org*** *Phytologia 106(2): 13-32 (June 20, 2024). ISSN 030319430.*

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The *Juniperus communis* L. complex is widely distributed throughout the northern hemisphere, with several infraspecific entities, morphologically different but genetically quite similar. In his description of the species Linné (1753) recognized the occurrence of infraspecific entities, describing three groups: a tree juniper, a shrub juniper and a minor mountain juniper. Along the 19th century three entities were differentiated in Europe. The first one included ascending to arborescent junipers with straight patent leaves. The second group included procumbent mountain junipers with incurved leaves. A third group was added in 1822, of procumbent mountain junipers with straight leaves, described from Sicilian specimens. According to the different authors, these groups were considered species, varieties or subspecies, with a plethora of names, among which *sibirica*, *saxatilis*, *nana*, *montana* or *alpina* dominate for the first group, *communis, suecica* or *vulgaris* for the second, and *hemisphaerica* for the latter.

The profuse taxonomy of *J. communis* is due to its variability and the fact that the characters used in its differentiation are not decisive individually, although they may be consistent when considered together. The groups of junipers have been separated for their leaves of a somewhat different width or length to the "type" juniper, incurved or straight, and with seed cones of greater or lesser size; but in all cases it is possible to find a continuous range of shapes and dimensions between the extremes. In addition, the habit of the plant is a constant in the descriptions, although it is strongly influenced not only by climate and wind exposure, but also by human action. Adams and Schwarzbach (2012) indicate that the separation between *J. communis* var. *communis* and *J. communis* var. *saxatilis* Pallas in Europe and Central Asia is largely based on the arboreal versus shrubby habit, although many populations include individuals ranging from trees to decumbent shrubs. Arborescent junipers are common in some regions, but rare in others, such as the Mediterranean, where a very slow growth is combined with a secular anthropic pressure. The variability of *J. communis* is evident within a population, with a large number of morphotypes, but also at the individual level, with branches with leaves of different curvature, width and length (Enríquez-de-Salamanca 2017).

Traditionally the juniper entities were defined by the coincidence of morphological characters, highly variable as seen. It is possible to find specimens coincident with one of the three groups cited, but also a large number of intermediate forms. This has been interpreted as introgressions or hybrids, although sometimes the presence of all three groups has been cited even in the same locality. A second stage in the taxonomy of junipers was based on the use of chemistry, differentiating entities according to their composition (e.g., Adams 1970, 1998; Barbero et al. 1990; Caramiello et al. 1995; Filipowicz et al. 2006; Mustafa et al. 2016). The most recent approaches apply DNA, being increasingly more precise; most of the work was conducted by R.P. Adams and collaborators (e.g., Adams et al. 2011, 2020; Adams and Schwarzbach 2012; Adams and Espeut 2020). One of these studies (Adams et al. 2003) identified two major groups of *J. communis*, one from America and the other from Eurasia. The validity of *Juniperus hemisphaerica* J. Presl & C. Presl, one of the three groups described above, has been discussed; initially rejected based on DNA studies (Adams and Pandey 2003; Adams 2011), it was recovered in subsequent studies (Adams and Schwarzbach 2012).

There is no consensus among botanists in the taxonomic treatment of this group, due to discrepant criteria when considering species, subspecies, or varieties, and even different criteria for what constitutes a variety or a subspecies. Clausen (1941) indicated that the variety was the oldest infraspecific unit, first used by Linnaeus, and subspecies were proposed latter to separate the most important variations within a species, often correlated with geographical, ecological or physiological isolation. According to this author using the term variety for those important variations is to assign a new meaning to it. Kapadia (1963) considered that the rank below the species is the variety, and if different varieties show patterns of affinities, then they should be grouped in subspecies. Hamilton and Reichard (1992) pointed out that despite attempts to differentiate subspecies and varieties, they are largely equivalent in practice, and that taxonomists use the former (especially in the United States) or the latter (especially in Europe) in the same sense. Hamilton (1995) pointed out that the choice between subspecies and varieties is more related to the taxonomists' country of origin than to the biology of the plants. The Botanical Code (Turland et al. 2018) indicates that the main rank below the species is the variety, but accepts that subspecies may be defined without the existence of varieties.

Considering the *J. communis* groups as independent species is increasingly uncommon, even more so after genetic studies have showed their similarity. One of the most influential works in Europe that defended the treatment as independent species was Flora d'Italia (Pignatti 1982), which recognized *J. communis*, *J. nana* Willd. and *J. hemisphaerica*. Franco (1962) and Franco and Rocha (1968) proposed a subspecific treatment, which the first author maintained in Flora Europaea (Franco 1964) and in Flora iberica (Franco 1986); the same approach was followed in other European floras, such as that of the British Isles (Thomas et al. 2007), Eastern Andalusia (Pérez-Latorre et al., 2011) or France (Tison and Foucault 2014). In contrast, Adams (1993) considered these groups varieties in Flora of North America, and in all his subsequent profuse work. The varietal treatment was also chosen by Farjon (2001, 2005), Farjon et al. (2014) or Schulz et al. (2005). There are, thus, specific, subspecific and varietal schools that do not agree. There is a morphological differentiation at the extremes of the "*saxatilis*" and "*communis*" groups, but not in the intermediate specimens; genetic differentiation is scarce; and the groups have a different ecology, with creeping junipers growing mainly in mountain areas. There are valid arguments supporting both subspecific and varietal differentiation. This paper does not aim to resolve this taxonomic conflict, but it is not possible to approach the taxonomy of *J. communis* without adopting an *a priori* position on the treatment of the infraspecific rank. We have opted for the varietal treatment, consistent with the scarce genetic differentiation of the infraspecific entities, and which seems to arouse greater consensus at present.

This paper focuses on two hybrids between the described groups. The first, originally named *J. intermedia* Schur., has been widely cited throughout Europe for a century and a half. We vindicated the validity of this hybrid, proposing a typification, which is currently missing. There are several names applied

to parts of the *J. communis* complex that have been misinterpreted, and that should be referred to this nothotaxa. The second is a hybrid recently reported, in 2020, but not properly described.

MATERIALS AND METHODS

The starting point for this work was the identification, in Spain, of frequent specimens with intermediate characters between *J. communis* var. *communis* and *J. communis* var. *saxatilis* (*J. communis* subsp. *alpina* (Suter) Čelak in Spanish floras, such as Franco, 1986 and Pérez-Latorre et al., 2011), growing in contact areas between both varieties. Traditionally included in *J. communis* var. *hemisphaerica* (J. Presl & C. Presl) Parl., we attributed them to *J. intermedia* Schur. (Enríquez-de-Salamanca 2017), a hybrid between these two varieties. Numerous references to this hybrid were detected from its description to the present day, along with mentions of introgressions or hybridizations between the parental varieties. However, it has been largely ignored in the floras (except Tison and Foucault 2014) and in the main botanical databases, where it is considered a synonym of some of the main varieties.

To analyze the distribution of this nothotaxon, an initial search for herbarium material attributed to *J. intermedia* (as a species, subspecies or variety) was carried out. This search confirmed the presence of intermediate specimens between *J. communis* var. *communis* and *J. communis* var. *saxatilis* throughout Europe. Analyzing historical descriptions of infraspecific taxa of *J. communis*, we detected several names (mainly from the 19th century but some of them later), whose descriptions pointed to this nothovariety. In some cases the currently established synonymies were not consistent with the descriptions. Herbarium materials referred to these taxa were consulted, online or in person, searching wherever possible for types, to determine their attribution. As a result, eight of these names, published between 1841 and 2002, have been proposed as synonyms of *J. communis* nothovar. *intermedia*. In this search for herbarium material, we detected frequent attributions of specimens to infraspecific taxa of *J. communis* that were not consistent with the original descriptions. Especially, throughout Europe disparate specimens of juniper have been included in *J. hemisphaerica* (as a species, subspecies or variety), frequently with little relation to what was originally described. The *J. intermedia* described by Schur in 1851 lacks a type. During the searches for material, a specimen collected by Sanio in 1852 was located, and has been proposed as a neotype. After these synonyms were identified, a global search of academic literature, grey literature, herbarium sheets, and databases was carried out to establish the distribution of the nothotaxon.

During these searches, reference was also found to a hybrid between *J. communis* var. *hemisphaerica* and *J. communis* var. *saxatilis*, reported in 2020 for southern Spain and France. The original publication lacks a description and typification, but the material cited by the authors has been located in the herbarium of Baylor University. We have done an effective typification.

TAXONOMIC TREATMENT

Juniperus communis nothovar. ***intermedia*** (Schur) Sanio, Deutsche Bot. Monatsschr. 4: 51 (1883) ≡ ***Juniperus communis*** var. ***communis*** × ***Juniperus communis*** var. ***saxatilis*** Pallas. Basionym: *Juniperus intermedia* Schur, Verh. Mitth. Siebenbürg. Vereins Naturwiss. Hermannstadt 10: 169 (1851); *ind. loc.*: [Romania] Transylvania (Schur, 1851); [Romania, Southern Carpathians] Fogarascher Alpen aus einer Höhe von 3000 (Fuss, 1851); type unknown or not described. **Neotype** (designated here): [Poland, Warmia–Masuria] Lyck [Ełk], Schedlisken [Siedliska] Berge auf einer mit Gebüsch bewachsenen Wiese, *C.G. Sanio*, 6 October 1852, AMD 126030 (Fig. 1)

=*J. communis* var. *montana* Aiton *sensu* Spach, Ann. Sci. Nat., Bot. 12: 301 (1841), *nom. illeg.*, syn. nov.

=*J. nana* var. *montana* (Aiton) Endl., Syn. Conif. 14 (1847), *nom. illeg.*, syn. nov.

=*J. pygmaea* K.Koch, Linnaea 22(3): 302 (1849), syn. nov. = *Juniperus communis* subsp. *pygmaea* (K.Koch) Imkhan., Novosti Sist. Vyssh. Rast. 27: 10 (1990), syn. nov.

=*J. depressa* Steven, Bull. Soc. Imp. Naturalistes Moscou 30(2): 398 (1857), *nom. invalid.*, syn. nov. Lectotype (designated by Shevera and Fedoronchuk 2011): Tauria [Crimea], 1813, Steven, H 1505535; syntype LE 01078132 = *J. communis* var. *depressa* (Steven) Boiss. Fl. Orient. 5: 707 (1884), *nom. illeg.*, syn. nov.

=*J. communis* subsp. *intermedia* (Schur) K. Richt., Pl. Eur. 1: 6 (1890) Pallas

=*J. sibirica* var. *montana* (Aiton) Beck, Blätt. Vereins Landesk. Nieder-Österreich 1890: 78 (1890), *nom. illeg.*, syn. nov.

=*J. communis* var. *castellana* Sennen & Pau, Bull. Acad. Inst. Géogr. Bot. 229: 466 (1908), syn. nov.; *ind. loc.*: entre Vitoria et Miranda de Ebro; Lectotype: [Spain, Burgos] Castille, Bugedo, garrigues, Apr 1906, *Sennen & Hno. Elías*, BC 59409; syntypes: MA 01 2773, MA 02 2773, MA 01 2776, MA 02 2776; paratypes: MA 01 2775, BC 59408

=*J. communis* subsp. *communis* var. *intermedia* (Schur) Sanio in Bolòs & Vigo, Fl. Països Catalans 201 (1984), *nom. illeg.*

=*J. communis* nothosubsp. *guadarramica* Rivas Mart., Itinera Geobot. 15(2): 703 (2002) *nom. invalid.*, syn. nov.

=*J. communis* nothosubsp. *intermedia* (Schur) Enríquez de Salamanca, Fl. Montib. 69: 33 (2017), *nom. illeg.*

Figure 1. Neotype of *Juniperus communis* nothovar. *intermedia* (Schur) Sanio. AMD126030 (Photograph: Naturalis Biodiversity Center, licensed under CC0).

Description: Intermediate between parents. Shrub decumbent, rarely erect, with ascendent branches. Leaves 10-20 x 1-2 mm, patent to slightly subimbricate, with curved base. Fertile hybrid; seed cones ovoid or globose, 6-10 mm, dark blue, often pruinose. It differs from *J. communis* var. *communis* by the curved base of the leaves, often with a slightly subimbricate distribution. It differs from *J. communis* var. *saxatilis* in its decumbent or ascending shape, never creeping or prostrate, and in its slightly imbricate leaves, with a tendency to be patent.

Distribution (Fig. 2)**: ALBANIA: Shkodër, Kukër,** Janković (1960, as *J. intermedia*). **ANDORRA:** MA918747, GBIF (2023, as *J. hemisphaerica*), GBIF (2023, as *J.c.* subsp. *communis* var. *intermedia*). **ARMENIA:** B100469800, MA742249, UTC239185, GBIF (2023, as *J. depressa*), MW642518, MW642519, MW0642522 to MW0642531, MDH (2023, as *J. depressa et J. hemisphaerica*), P01636676, GBIF (2023, as *J. oblonga*), Boissier (1884, as *J.c.* var. *depressa*), Grossgejm (1928, 1949, as *J. depressa*), Takhtajan (1954, as *J. depressa*), Takhtajan (2003, as *J.c.* subsp. *hemisphaerica*). **AUSTRIA: Alps** [unprecise] Vierhapper and Handel-Mazetti (1905, as *J. intermedia*), Elwes and Henry (1912, as *J.c.* var. *intermedia*), Vidakovic (1991, as *J.c.* var. *intermedia*); **Kärnten,** Hruby (1916, as *J. intermedia*); **Niederösterreich,** LI100297221, GBIF (2023, as *J. intermedia*); **Steiermark**, Hayek (1901, 1904, as *J. intermedia*), Schittengruber (1960, as *J. intermedia*); **Tirol**, Kerner (1889, as *J. intermedia*), MA171891, RJB (2023, as *J. intermedia*), CLF142346, LY670275, GBIF (2023, as *J. intermedia*), ReColNat (2023, as *J. intermedia*). **AZERBAIJAN:** MW0642532 to MW0642541, MDH (2023, as *J. depressa et J. hemisphaerica*), Boissier (1884, as *J.c.* var. *depressa*), Grossgejm (1928, 1949, as *J. depressa*), Takhtajan (2003, as *J.c.* subsp. *hemisphaerica*). **BULGARIA: Yugozapaden**, Rechinger (1933, as *J. intermedia*), Komarov and Il'in (1968, as *J. depressa*). **BOSNIA-HERZEGOVINA: Federation of Bosnia and Herzegovina**, Siljak-Yakovlev et al (2010, as *J. intermedia*), Operta et al (2018, as *J. intermedia*); **Republika Srpska**, Fukarek (1969, as *J. intermedia*). **CROATIA: Lika-Senj**, SVNP (2023, as *J. intermedia*); **Primorje-Gorski Kotar**, Vidakovic (1991, as *J.c.* var. *intermedia*), ZAHO58197 to ZAHO58200, FCD (2023, as *J. intermedia*). **CZECHIA: Moravskoslezský,** NATURALIS (2024, as *J. nana* var. *intermedia*); **Olomoucký**, Oborny (1885, as *J. intermedia*), BRNU593502, JACQ (2023, as *J. intermedia*); **Sudetes** [unprecise] Vidakovic (1991, as *J.c.* var. *intermedia*). **FINLAND: Lapland**, Elwes and Henry (1912, as *J.c.* var. *intermedia*). **FRANCE: Auvergne-Rhône-Alpes**, CLF142345, UniVegE (2023, as *J. intermedia*), Tison and Foucault (2014, as *J.c.* nothosubsp. *intermedia*), CBNA (2023, as *J.* × *intermedia*), BIODIV'AURA ATLAS (2023, as *J.c.* subsp. *intermedia*), INPN (2023, as *J.c.* nothosubsp. *intermedia*); **Occitanie**, AMD126028, GBIF (2023, as *J. communis* var. *intermedia*), MA233850, P41888, GBIF (2023, as *J.c.* subsp. *hemisphaerica*), Tison and Foucault (2014, as *J.c.* nothosubsp. *intermedia*), INPN (2023, as *J.c.* nothosubsp. *intermedia*); **Provence-Alpes-Côte-d'Azur**, P00044293, GBIF (2023, as *J.c.* subsp. *hemisphaerica*), Barbero (1981, as *J. intermedia*), Lebreton et al (2013, as *J.* × *intermedia*), Tison and Foucault (2014, as *J.c.* nothosubsp. *intermedia*), CBNA (2023, as *J.* × *intermedia*), Lamaison (2023, as *J.c.* nothosubsp. *intermedia*), INPN (2023, as *J.c.* nothosubsp. *intermedia*). **GEORGIA:** MO1328390, MO1328511, NY19440, NY804112, GBIF (2023, as *J. hemisphaerica*), MW642459, MW642503 to MW642517, MDH (2023, as *J. depressa et J. hemisphaerica*), BR32183573, MA576696, WAG1733039, GBIF (2023, as *J. oblonga*), Boissier (1884, as *J.c.* var. *depressa*), Grossgejm (1928, 1949, as *J. depressa*), Gagnidze (1971, as *J. depressa*), Takhtajan (2003, as *J.c.* subsp. *hemisphaerica*), Gagnidze (2005, as *J. hemisphaerica*). **GERMANY: Bayern (Oberbayern)**, Michiels (1996, as *J.c.* var. *intermedia*); **Saarland**, Delattinia (2023, as *J. intermedia*); **Sudetes (Sachsen)** [unprecise] Vidakovic (1991, as *J.c.* var. *intermedia*); **Alps** [unprecise] Elwes and Henry (1912, as *J.c.* var. *intermedia*), Vidakovic (1991, as *J.c.* var. *intermedia*). **GREECE:** Boissier (1884, as *J.c.* var. *depressa*); **C Greece**, LD1291214, GBIF (2023, as *J.c.* subsp. *hemisphaerica*); **Epirus**, LD1286352, LD1298423, GBIF (2023, as *J.c.* subsp. *hemisphaerica*); **Macedonia**, P01621070, RECOLNAT (2023, as *J. depressa*); **Peloponnese**, MA871954, GBIF (2023, as *J.c.* subsp. *hemisphaerica*), LY670708, LY670710, P01636615, RECOLNAT (2023, as *J. depressa*); **Thessaly**, LD1215867, LD1281442, LD1284863, LD1293488, GBIF (2023, as *J.c.* subsp. *hemisphaerica*); **W Greece**, LY670269, LY670271, LY670272, RECOLNAT (2023, as *J. hemisphaerica*).

IRAN: Tabriz, Komarov and Il'in (1968, as *J. depressa*), Hamidipour *et al.* (2011, as *J. oblonga*), Kasaian *et al.* (2011, as *J. oblonga*). **IRELAND:** [unprecise] Clapham et al (1987, intermediate forms between *J.c.* subsp. *communis* and *J.c.* subsp. *alpina*), Stace (2010, intermediates between *J.c.* subsp. *communis* and the other two subspecies [*J.c.* subsp. *hemisphaerica* and *J.c.* subsp. *nana*]). **ITALY:** Conti et al (2005, as *J.c.* var. *intermedia*); **Abruzzo,** Pl@ntNet-1008345216, GBIF (2023, as *J. communis*); **Aosta Valley**, Peyronel and Dal Vesco (1971, as *J.c.* var. *intermedia*), Barbero (1981, as *J. intermedia*), Caramiello et al (1995, as *J. intermedia*); **Firuli-Venezia-Giulia**, Pl@ntNet-1007496890, Pl@ntNet-1004197201, GBIF (2023, as *J. communis*); **Lombardia,** Inaturalist-168725251, GBIF (2023, as *J. communis*); **Piedmont**, Caramiello et al (1995, as *J. intermedia*); **Trentino-Alto Adige**, Ribbons (1972, as *J. intermedia*), Meurer (1979, as *J. intermedia*); **Veneto**, Inaturalist-97925794, GBIF (2023, as *J. c.* var. *saxatilis*); **Alps** [unprecise] Elwes and Henry (1912, as *J.c.* var. *intermedia*), Vidakovic (1991, as *J.c.* var. *intermedia*). **KOSOVO: Gjakova**, Janković (1960, as *J. intermedia*), Dauti et al (2003, as *J. intermedia*); **Prizren,** Janković et al (1987, as *J.c.* var. *intermedia*), **MONTENEGRO: Pljevlja, Žabljak,** Petrović et al, (2019, as *J.c.* var. *intermedia*). **NORTH MACEDONIA: Polog**, Janković et al (1987, as *J.c.* var. *intermedia*), Nikolovski (1970, as *J. intermedia*), Vidakovic (1991, as *J.c.* var. *intermedia*), Simovski (2013, 2015, as *J. intermedia*); **Pelagonia**, Em (1978, as *J. intermedia*), Acevski and Simovski (2009, as *J.c.* var. *intermedia*). **NORWAY: Nord-Norge** [unprecise] Rikli (1936, as *J.c.* var. *intermedia*). **POLAND: Lower Silesia**, Boratyński (1985, as *J.c.* subsp. *communis* var. *intermedia*); **Silesia**, RB637945, GBIF (2023, as *J. intermedia*), Browicz and Zieliński (1974, as *J.c.* var. *intermedia*); **Warmia-Masuria**, AMD126030, GBIF (2023, as *J. communis* var. *intermedia*), Sanio (1883, as *J.c.* var. *intermedia*); **Sudetes** [unprecise] Vidakovic (1991, as *J.c.* var. *intermedia*). **ROMANIA: Centru,** MO883899, GRSciColl (2023, as *J. communis* var. *intermedia*), Simonkai (1886, as *J. intermedia*), Römer (1911, as *J. intermedia*), Pop (1939, as *J. intermedia*), Claudiu (2012, as *J.c.* var. *intermedia*); **Nord-Est**, Pop (1939, as *J. intermedia*), Chifu et al (2006, as *J.c.* var *intermedia*); **Nord Vest,** Gyula (1915, as *J. intermedia*), Pop (1939, as *J. intermedia*), Moldovan (1971, as *J.c.* var. *intermedia*), Claudiu (2012, as *J.c.* var. *intermedia*); **Sud-Muntenia,** Schur (1851, as *J. intermedia*); **Transilvania** [unprecise], Richter (1890, as *J. communis* subsp. *intermedia*); **Carpathians** [unprecise] Vidakovic (1991, as *J.c.* var. *intermedia*). **RUSSIA: North Caucassian**, NSK66161, NSK66174, LY670712, LY670713, LY670715, GBIF (2023, as *J. depressa*), MW638299, MW642463 to MW642465, MW642479 to MW642501, MDH (2023, as *J. depressa et J. hemisphaerica*), BR32183511, BR32183498, BR32183559 , NSK66160, GBIF (2023, as *J. oblonga*), Grossgejm (1928, 1949, as *J. depressa*), Takhtajan (2003, as *J.c.* subsp. *hemisphaerica*), Murtazaliev (2009, as *J. hemisphaerica*); **North of Russia** [unprecise], Rikli (1936, as *J.c.* var. *intermedia*); **Southern**, MW642460 to MW642462, MW642470 to MW642472, MW642502, MW1011806, MDH (2023, as *J. depressa et J. hemisphaerica*). **SERBIA: Pirot,** Jovanović et al. (1992, as *J. intermedia*), Marković et al. (2009, as *J.c.* var. *intermedia*); **Nisava, Raska**, Lakušić and Lakušić (2011, as *J.c.* subsp. *communis* var. *intermedia*). **SLOVAKIA: Banská Bystrica**, Jasičová (1966, as *J.c.* var. *intermedia*); **Prešov**, Hruby (1934, as *J. intermedia*), Jasičová (1966, as *J.c.* var. *intermedia*); **Žilina**, Jasičová (1966, as *J.c.* var. *intermedia*), Kučera (2019, as *J.* × *intermedia*); **Carpathians** [unprecise] Vidakovic (1991, as *J.c.* var. *intermedia*). **SLOVENIA: Zahodna Slovenija,** ZA56104, FCD (2023, as *J. intermedia*); **Vzhodna Slovenija**, Accetto (2001, as *J.c.* var. *intermedia*). **SPAIN: Aragón**, BIO10283, GBIF (2023, as *J.c.* var. *intermedia*), MA2738, MA2770, MA326131, GBIF (2023, as *J.c.* subsp. *hemisphaerica*), Gandoger (1896, 1917, as *J. intermedia*), Rivas Goday and Borja (1961, intermediate specimens between *J.c.* subsp. *communis* and *J.c.* subsp. *alpina*), Rivas Martínez et al (2002, as *J. intermedia*); **Basque Country**, LISI001583, GBIF (2023, as *J.c.* subsp. *hemisphaerica*); **Castile-León**, MA2773, MA2775, MA2776, MA2777, RJB (2023, as *J.c.* var. *castellana*), MA2720, MA228430, MA358365, MA567959, MA639977, MA656817, MA7547871, GBIF (2023, as *J.c.* subsp. *hemisphaerica*), Gandoger (1896, 1917, as *J. intermedia*), Enríquez-de-Salamanca (2017, as *J.c.* nothosubsp. *intermedia*); **Castile-La Mancha**, MA2733, MA2734, MA809596, GBIF (2023, as *J.c.* subsp. *hemisphaerica*), Enríquez-de-Salamanca (2017, as *J.c.* nothosubsp. *intermedia*); **Catalonia**, HGI11753, HGI11754, GBIF (2023, as *J.c.* subsp. *communis* var. *intermedia*), Carreras et al (1995, as *J.c.* var *intermedia*), Soriano (1998, as *J.c.* var. *intermedia*), Ninot and Carrillo (2019, as *J.c.* var. *intermedia*), Bolòs and Vigo (1984, as *J.c.* subsp. *communis* var. *intermedia*), GBIF (2023, as *J.c.* var. *intermedia*); **Madrid**,

MA2718, MA-2763, GBIF (2023, as *J.c.* subsp. *hemisphaerica*), Enríquez-de-Salamanca (2017, as *J.c.* nothosubsp. *intermedia*); **Navarra**, MA809594, GBIF (2023, as *J.c.* subsp. *hemisphaerica*). **SWEDEN: Norland** [unprecise] Rikli (1936, as *J.c.* var. *intermedia*). **SWITZERLAND:** AMD126027, GBIF (2023, as *J. communis* var. *intermedia*), Erb (1897, as *J. intermedia*), Brockmann-Jerosch (1907, as *J.c.* var *intermedia*), Brunies (1928, as *J.c.* var. *intermedia*), Braun-Blanquet et al (1954, as *J. intermedia*), Theurillat et al (2021, as *J.c.* var. *intermedia*); **Alps** [unprecise] Elwes and Henry (1912, as *J.c.* var. *intermedia*), Vidakovic (1991, as *J.c.* var. *intermedia*). **TÜRKIYE: Aegean**, **Central Anatolia**, **Eastern Anatolia** and **Marmara**, Davis (1965, intermediate between *J.c.* subsp. *hemisphaerica* and subsp. *nana*). **UNITED KINGDOM:** [unprecise] Clapham et al (1987, intermediate forms between *J.c.* subsp. *communis* and *J.c.* subsp. *alpina*), Stace (2010, intermediates between *J.c.* subsp. *communis* and the other two subspecies [*J.c.* subsp. *hemisphaerica* and *J.c.* subsp. *nana*]); **England**, L1187750, GBIF (2023, as *J. communis* subsp. *intermedia*); **Scotland**, E00853899, RBGE (2023, as *J.c.* var. *intermedia*), Bennett (1893a,b, as *J. intermedia*), McNeill (1910, as *J.c.* var. *intermedia*), Matthews (1930, as *J.c.* var. *intermedia*), Hayward and Druce (1930, as *J.c.* var. *intermedia*), Watt (1931, as *J.c.* var. *intermedia*), Heslop (1937, as *J.c.* var. *intermedia*), Muirhead (1961, as *J.c.* var. *intermedia*), Kenneth (1971, as *J.c.* var. *intermedia*), Kenworthy (1976, intermediate forms between *J.c.* subsp. *communis* and subp. *nana*), Clapham et al (1987, intermediate forms between *J.c.* subsp. *communis* and *J.c.* subsp. *alpina*). **UKRAINE: Crimea**, LY0670711, GBIF (2023, as *J. depressa*), MW0600425 to MW0600456, MW0631697, MDH (2023, as *J. depressa et J. hemisphaerica*), BR20384883, NSK0066164, P01621071, GBIF (2023, as *J. hemisphaerica*), Boissier (1884, as *J.c.* var. *depressa*), Komarov and Il'in (1968, as *J. depressa*); ***Zakarpattia***, MHA0004442, GBIF (2023, as *J. sibirica*), MHA0000689, GBIF (2023, as *J. communis*); **Carpathians** [unprecise] Vidakovic (1991, as *J.c.* var. *intermedia*).

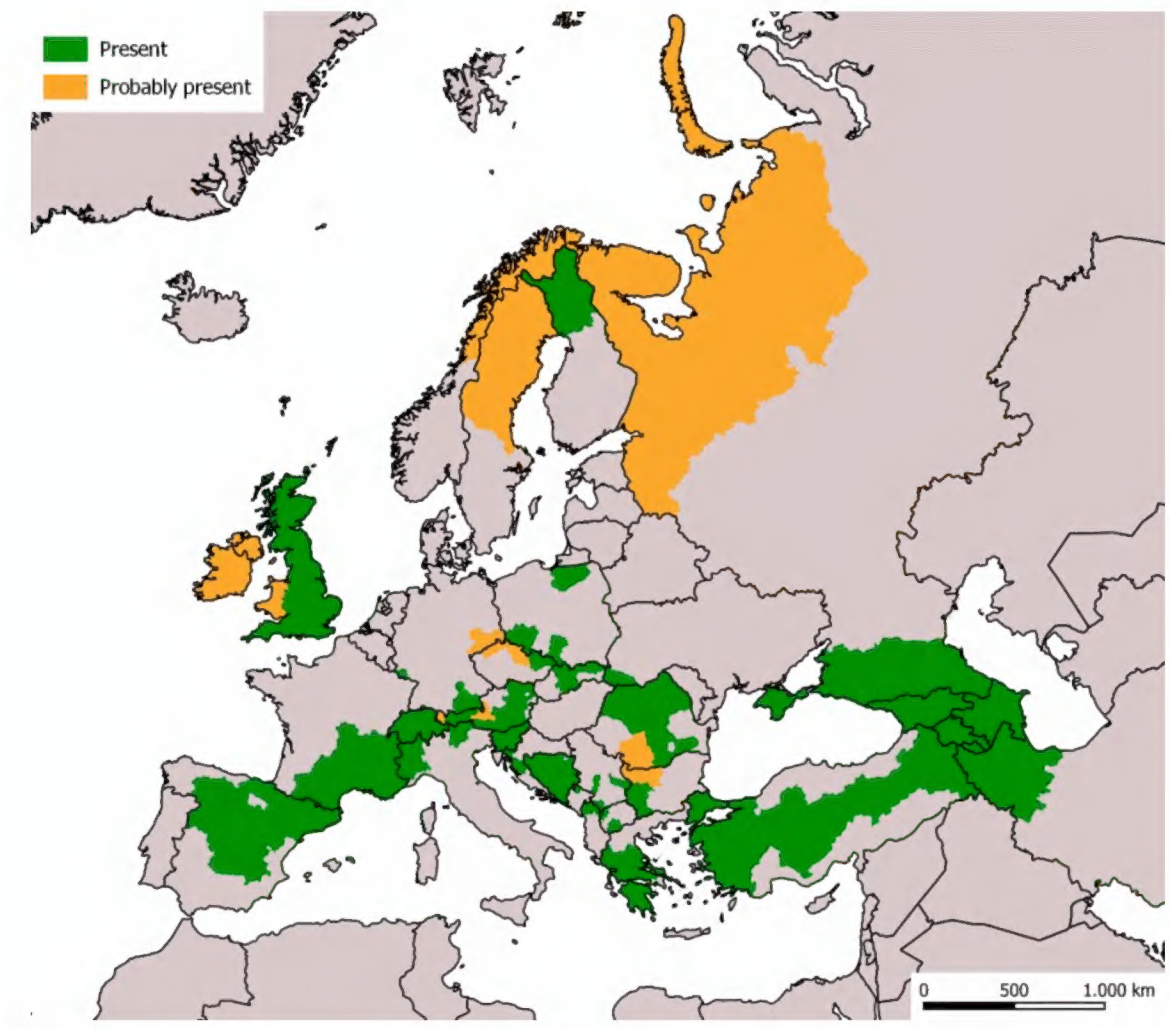

Figure 2. Distribution of *Juniperus communis* nothovar. *intermedia.*

Discussion: In the Bulletin of the Transylvanian Society of Natural Sciences of Sibiu in October 1851, Schur (1851) described *J. intermedia* as a form of *J. nana* with long, patent leaves. In the following bulletin (November 1851), Carl Fuss compiled news from the Society (Fuss 1851), indicating that Schur showed wild Transylvanian specimens of *J. communis* and *J. nana*, including an intermediate form from the Fagaras Mountains named *J. intermedia*. In the Flora of Transylvania, Mihály Fuss (1866) includes Schur's species, with no indication of its hybrid origin, but with a Latin description.

Sanio (1883) proposed *J. intermedia* as a variety of *J. communis*, without citing its hybrid origin, while Richter (1890) proposed it as a subspecies, indicating that it was a hybrid. According to the botanical code (Turland et al. 2018), for homonymy and synonymy purposes both the multiplication sign and the prefix *notho-* are ignored, so both combinations are valid as nothovariety and nothosubspecies.

There is no known type for this hybrid, nor has any original material or illustration by Schur been located. There is a specimen collected by Carl Gustav Sanio at Lyck, East Prussia (nowadays Ełk, Poland), in 1852 (AMD 126030), just one year after Schur's description, and labeled "*Juniperus communis* L. var. *intermedia* Schur". It was not until 31 years later that Sanio (1883), in his work on the varieties of *J. communis* in the Lyck flora, validly published the name *J. communis* var. *intermedia* (Schur) Sanio. In the absence of original material from Schur, we have proposed as neotype this sheet of Sanio, which is also the material that supports the varietal combination proposed by this author, and which is still the valid name for the nothovariety.

Drobow (1941) described *J. intermedia* Drobow, but it was an illegitimate name, synonym of *J. semiglobosa* Regel, and not the same as *J. intermedia* Schur. Jasičová (1966) considered *J. intermedia* and *J. communis* var. *intermedia* (Schur) Sanio, synonyms of *J. communis* × *J. nana*. Bolòs and Vigo (1984) proposed the combination *J. communis* subsp. *communis* var. *intermedia* (Schur) Sanio, criterion also applied by Boratyński (1985) and Lakušić and Lakušić (2011), but it is an illegitimate name. Adams (2008), in the second edition of his "Junipers of the world" considered *J. intermedia* as a synonym of *J. communis* var. *depressa* Pursh, an erroneous synonymy because *J. communis* var. *depressa* Pursh refers to a juniper from North America, while Schur's description pertained to plants from Romania. However, in the fourth edition of his book (Adams 2014) the Schur's name was considered a synonym of *J. communis* var. *communis*, a criterion also followed by Farjon et al. (2014).

Since Schur's description of *J. intermedia* in 1851, numerous references to this taxon –as a species, subspecies, variety, or hybrid– have been recorded over a century and a half from most of Europe. The taxon began to be cited regularly from the 1880s onwards, with the highest number of citations recorded in recent years. A main source of records is Central Europe. Citations are also abundant in Western and Southern Europe, with France having the highest percentage of recent citations. In this country Lebreton et al. (2000) considered a hybridization-introgression between *J. communis* subsp. *communis* and *J. communis* subsp. *nana* in the Pre-Alps and Massif Central, which they included in this taxon. Tison and Foucault (2014) considered the French citations of *J. communis* subsp. *hemisphaerica* to be confused with *J. communis* nothosubsp. *intermedia*. In Spain, Enríquez-de-Salamanca (2017) analyzed the populations of *J. communis* from the Central Range, where *J. communis* subsp. *communis*, *J. communis* subsp. *alpina* and *J. communis* subsp. *hemisphaerica* were cited; the presence of the latter was ruled out, and these specimens were included in part in *J. communis* subsp. *communis* and in others in *J. communis* subsp. nothosubsp. *intermedia*. Another area with a significant number of records is the Balkan Peninsula. In Eastern Europe and the Caucasus references to *J. intermedia* are fewer because most specimens of this hybrid have been named *J. depressa* Steven or *J. hemisphaerica*. In the British Isles, references to *J. intermedia* mainly come from Scotland, while at the regional level there are several references to intermediate forms between *J. communis* subsp. *communis* and *J. communis* subsp. *nana*, without being given any name. Finally, in Scandinavia there are very few records.

Several names proposed within the *J. communis* complex seem to refer to this nothotaxon:

Juniperus depressa Steven.

Pursh (1813) described *J. communis* var. *depressa* Pursh from Canada and the western part of New York. A few decades later, Steven (1857) described *J. depressa* Steven from Crimea, referring to something different. The Steven's description referred to an intermediate form between *J. communis* and *J. pygmaea* (both taxa present in the area), closer to the latter. The syntype (LE01078132) has long leaves, subimbricate, with slightly curved base, so we referred it to *J. communis* nothovar. *intermedia*. Boissier (1884) considered Steven's *J. depressa* to be a variety of *J. communis*, synonym with *J. oblonga* M. Bieb., *J. hemisphaerica*

and *J. pygmaea*, citing it in present-day Greece, Crimea, Georgia, Armenia, Azerbaijan and Iran. Many specimens collected in Crimea and Russia (and more rarely Greece) between 1860 and 2014 (preserved in the herbaria K, LY, MW, NSK and TU) were initially identified as *J. nana* (especially from 1910 to 1923), then as *J. depressa* Steven, and the most recent ones as *J. communis* subsp. *hemisphaerica*. In several sheets *J. depressa, J. pygmaea* and *J. hemisphaerica* were considered synonyms, as Boissier (1884) did. Kew sheets K000089226 from Crimea and K000089228 from Iran, labeled as *J. depressa*, were identified by R. P. Adams as *J. communis* var. *montana* (= *J. communis* var. *saxatilis*), an identification that aligns with our opinion. We have included in *J. communis* nothovar. *intermedia* some material from Crimea (LY0670711), Greece (LY0670708, LY0670710) and Russia (LY0670712, LY0670713, LY0670715, NSK0066161, NSK0066174). Most of these plants, including the species' syntype, have leaves that are incurved or subincurved, a character that sets them apart from *J. communis* var. *hemisphaerica* (as proposed by local botanists), and from var. *communis* (as proposed by Farjon et al. 2014). Adams (2008) considered *J. intermedia* as a synonym of *J. communis* var. *depressa* Pursh, a wrong synonymy, but later he changed this opinion (Adams 2014).

Juniperus communis / nana var. *montana auct. pl.*

The name "montana" was used in the botanical literature of the 18th and 19th centuries referring to varieties of *J. communis*, unevenly. Aiton (1789) applied the name *J. communis* var. *montana* Aiton (=*J. communis* var. *saxatilis*) to small mountain junipers. Neilreich (1859) considered *J. communis* var. *alpina* and *J. communis* var. *montana*, the later a synonym of *J. vulgaris* (= *J. communis* var. *communis*). Sometimes this name refers to specimens that do not belong to either *J. communis* var. *communis* or *J. communis* var. *saxatilis*. According to the original descriptions, we consider some of these names synonyms of *J. communis* nothovar. *intermedia*: *J. communis* var. *montana* Aiton *sensu* Spach (1841) [shrubby junipers 0.6–1.2 m tall], *J. nana* var. *montana* (Aiton) Endl. [ascending branches crowded of leaves, incurved–patent] and *J. sibirica* var. *montana* (Aiton) Beck.

Juniperus hemisphaerica auct. non J. Presl & C. Presl

The species was described by Jan and Carel Presl (Presl 1822) from Mount Etna in Sicily. The differences with *J. communis* were the prostrate shape, the leaves shorter and a little wider, bluish and patent, and the seed cones larger, of equal length or slightly shorter than the leaves. Parlatore (1868) considered this taxon as a variety of *J. communis*, while Nyman (1881) regarded it as a subspecies. Apart from the original region (Sicily), the taxon began to be cited in other areas, especially in Spain, but also in others such as France, Britain, Greece, Crimea or the Caucasus. However, identifications were often not consistent with the original. For example, in Crimea many specimens named *J. hemisphaerica* have little to do with the description of Presl (1882); we have included the revised material part of it in *J. communis* var. *saxatilis* and the rest in *J. communis* nothovar. *intermedia*. Tison and Foucault (2014) considered references to *J. communis* subsp. *hemisphaerica* in France confusion with *J. communis* nothosubsp. *intermedia*, and Enríquez-de-Salamanca (2017) shared the same opinion for Central Spain.

References to this taxon in Britain, specifically in the Lizard peninsula (England) and Pembrokeshire (Wales), are surprising due to its isolation. There seems to be a consensus among British authors and institutions that Lizard populations belong to *J. communis* var. *hemisphaerica*, but not those of Pembrokshire: Van der Merwe et al. (2000) and Preston et al. (2002) considered that they are *J. communis* subsp. *hemisphaerica*; Evans (2003) indicated that they showed intermediate characters between *J. communis* subsp. *nana* and *J. communis* subsp. *communis* (so they could be *J. communis* nothovar. *intermedia*); Stace (2010) considered that they are probably an extreme of *J. communis* subsp. *communis*; BSBI (2023) does not include this population in its map of the subspecies; BRC (2023) cites it in the text, but refers to the previous map, where it does not appear; and NMW (2023) indicates that recent genetic work indicates that it is *J. communis* subsp. *communis*. These localities are disjunct and isolated, making it

reasonable to assume that they represent an extreme of variation of *J. communis* var. *communis*, or specimens of *J. communis* nothovar. *intermedia*, rather than *J. communis* var. *hemisphaerica*. Genetic studies comparing these populations with those of Sicily and Sierra Nevada are needed.

Juniperus oblonga M. Bieb.

Marschall von Bieberstein (1808) described in his Flora taurico-caucasica *J. communis* and *J. oblonga*; the only important difference is that the former was subarborescent, while the latter was shrubby. POWO (2023) considers this name a synonym of *J. communis* var. *saxatilis*, but the original description indicates that the leaves are patent. The herbarium material attributed to *J. oblonga* that we have analyzed is diverse: we have considered some specimens as dense forms of *J. communis* var. *communis*, only one as *J. communis* var. *saxatilis*, and many of them as *J. communis* nothovar, *intermedia* (Armenia P01636676; Georgia BR32183573, MA576696, WAG1733039; Russia BR32183511, BR32183498, BR32183559, NSK66160).

Juniperus oblonga-pendula Loudon

Loudon (1838) includes in his description of this taxon a lamina, displaying long leaves, slightly incurved at the base, characters that suggest its possible inclusion in *J. communis* nothovar. *intermedia*. The original description is based on cultivated specimens, introducing a challenge for an accurate attribution. Koch (1863) points out that *J. oblonga-pendula* is probably the male plant of *J. oblonga*.

Juniperus communis var. *reflexa* f. *pendula* Carrière

Carrière (1855), in his Traité Général des Conifères, introduced a perplexing hierarchy of infraspecific taxa. He proposed a *pendula* form characterized by leaves measuring 15 mm in length, curved towards the branch and with two glaucous lines separated in the middle by a green line. The curved leaves lead to *J. communis* nothovar. *intermedia*, but the green vein is a character of *J.* × *souliei* Sennen (=*J. communis* var. communis × *J. oxycedrus* var. *oyxcedrus*).

Juniperus pygmaea K. Koch

Koch (1849) described *J. pygmaea* K. Koch from the Hemschin Mountains in Pertakrek (Hemşin, Rize province, Turkey), considering it as a species closely related to *J. nana* (= *J. communis* var. *saxatilis*), but prostrate or pyramidal, and with slightly curved leaves. This description is consistent with *J. communis* nothovar. *intermedia*. There is no known type, and according to Adams et al. (2014) the Koch collection was lost in a fire. Reviewing two herbarium specimens attributed to *J. communis* subsp. *pygmaea*, both collected in 1914 (Turkey NS0007551; unknown location L3774861), we observed the presence of straight leaves with a somewhat curved base, prompting their inclusion in *J. communis* nothovar. *intermedia*. The name *pygmaea* has been frequently used in Eastern Europe. Boissier (1884) considered it a synonym of *J. depressa* Steven, *J. oblonga* M. Bieb. and *J. hemisphaerica* Presl. Adams et al. (2014) examined junipers from Bulgaria and proposed *J. communis* f. *pygmaea* (K. Koch) R. P. Adams & A.N. Tashev. Their study concluded that it represents a shrubby form of *J. communis* var. *communis*, rather than *J. communis* var. *saxatilis*. They also noted specimens of *J. pygmaea* they considered to be of hybrid origin but did not specify the parentage. In a subsequent study in Azerbaijan (Adams et al. 2015), the researchers found discrepancies between what was called *pygmaea* there and their analysis in Bulgaria, so they opted to include them in *J. communis s.l.* While POWO (2023) considers *J. pygmaea* and the form proposed by Adams and Tashev, as synonyms of *J. communis* var. *saxatilis*, we posit that they are more appropriately synonyms of *J. communis* nothovar. *intermedia*.

Juniperus communis var. *castellana* Sennen & Pau

Sennen (1908) introduced the name *J. communis* var. *castellana* for creeping shrubs in northern Spain, characterized by shorter leaves compared to normal forms. IPNI (2023) does not include this name, but Flora iberica (2023) database considers it a synonym of *J. communis* subsp. *hemisphaerica*, aligning with the criteria of Joao Amaral Franco, who in 1961 determined some types accordingly. However, upon our examination of the types (BC59408, BC59409, MA 01 2773, MA 02 2773, MA 01 2776, MA 02 2776), we propose their inclusion in *J. communis* nothovar. *intermedia*.

Juniperus communis nothosubsp. *guadarramica* Rivas Mart.

Rivas-Martínez et al. (2002) proposed the name *J. communis* nothosubsp. *guadarramica* Rivas Mart. for a hybrid identified in Central Spain between *J. communis* subsp. *nana* and *J. communis* subsp. *hemisphaerica*. However, the presence of *J. communis* var. *hemisphaerica* in Central Spain has been both unproven and refuted (Enríquez-de-Salamanca 2017). Instead, the likely parentage involves *J. communis* var. *communis* and *J. communis* var. *saxatilis*. Therefore, *J. communis* nothosubsp. *guadarramica* should be considered a synonym of *J. communis* nothovar. *intermedia*.

Juniperus communis var. ***hemisphaerica*** (J. Presl & C. Presl) Parl. × ***J. communis*** var. ***saxatilis*** Pallas.
Holotype (designated here): Spain, Sierra Nevada, s. of Granada, N 37°06.170' W 3°24.515', 2100 m, *Robert P. Adams* 7194, 20 Oct. 1993, BAYLU043718 (Fig. 3, left); syntype (designated here) BAYLU043721 (Fig. 3, right).

Name: The International Code of Nomenclature (Turland et al. 2018, article H.10.B.1), indicates that "when contemplating the publication of names for hybrids between named infraspecific taxa, authors should carefully consider whether these names are really needed, bearing in mind that formulae, though more cumbersome, are more informative". There is no real need to assign a name to this hybrid, so only the hybrid formula has been proposed.

Description: This nothotaxon is difficult to diagnose by morphological characters alone. It has subincurved leaves, intermediate between the incurved and subimbricate leaves of *J. communis* var. *saxatilis* and the more or less patent leaves of *J. communis var. hemisphaerica*, although both parental taxa show wide variability. The only certainty for their differentiation is based on the genetic analysis conducted by Adams and Espeut (2020) and Adams et al. (2020). According to these authors, the plants from Sierra de Baza (*R. P. Adams* 15695, 15697, 15699, 15701) are heterozygous with nucleotides from both *J. communis* var. *hemisphaerica* and *J. communis* var. *saxatilis*, and the plants from Sierra Nevada (*Robert P. Adams* 7194, BAYLU043718; *Robert P. Adams* 7195, BAYLU043721), initially identified a *J. communis* var. *hemisphaerica*, have an A at site 1149 suggesting they are backcrossed from *J. communis* var. *saxatilis*, and they also have a T at cp petN-psbM site 305, common in *J. communis* var. *saxatilis* from the Pyrenees.

Distribution (Fig. 4): **FRANCE. Occitanie**, Pyrénées-Orientales, *Robert P. Adams* 15401 [no known registered herbaria], Adams and Espeut (2020), Adams et al. (2020). **SPAIN. Andalusia**, Granada, Adams and Espeut (2020), Adams et al. (2020): Sierra de Baza, *R. P. Adams* 15695, 15697, 15699, 15701 [no known registered herbaria]; Sierra Nevada, *Robert P. Adams* 7194 (BAYLU043718), *Robert P. Adams* 7195 (BAYLU043721), *S. Rojas Clemente*, MA153763 (genetic confirmation required), *J.M. Losa*, MA589195 (genetic confirmation required).

Figure 3. Types of *Juniperus communis* var. *hemisphaerica* × *J. communis* var. *saxatilis*. Left: Holotype, BAYLU043718. Right: Syntype, BAYLU043721 (Photographs: Baylor University Herbarium, licensed under CC0)

Discussion: Adams and Pandey (2003) examined RAPDs from *J. communis* var. *hemisphaerica* from its type locality (Mt. Etna, Sicily) and found it to be very similar to typical *J. communis* var. *communis* from Sweden, leading Adams (2011) to consider *J. communis* var. *hemispherica* as a synonym of *J. communis* var. *communis*. Similarly, Vargas (2003) found identical *trnL-trnF* sequences in specimens of these infraspecific taxa. However, subsequent research (Adams and Schwarzbach 2012) suggested that DNA data supported the recognition of *J. communis* var. *hemisphaerica* in plants from Mt. Etna in Italy and Sierra Nevada in Spain. Although the shapes of these plants from Sierra Nevada were dissimilar to those from Sicily, their ITS DNA was identical (Adams and Espeut 2020). Adams and Espeut (2020) identified hybrids between *J. communis* var. *hemisphaerica* and *J. communis* var. *saxatilis* in Southern Spain and the French Pyrenees but did not provide proper description and typification. Adams et al. (2020) located more of these hybrids in Southern Spain.

In the French Pyrenees, Adams and Espeut (2020) identified one specimen (*R.P. Adams* 15401) as a hybrid between *J. communis* var. *hemisphaerica* and *J. communis* var. *saxatilis*, displaying genetic markers indicative of both parents, specifically 3 SNPs from the former and the indel and 2 SNPs from the later. However, in this area only one parent, *J. communis* var. *saxatilis*, has been confirmed to be present. These authors suggested either the possibility of *J. communis* var. *hemisphaerica* growing in the region, a

proposition that was refuted by Lebreton et al. (2000) and Tison and Foucault (2014), or alternatively the hypothesis of pollen transfer over long distances. Further genetic studies are required to confirm the presence of this hybrid or discover the second parent. Consequently, this specimen has not been considered as a type for the hybrid.

Figure 4. Distribution of *Juniperus communis* var. *hemisphaerica* × *J. communis* var. *saxatilis*

In Granada (Spain) both parents are present, although their ecology is different, with *J. communis* var. *saxatilis* growing in siliceous areas, and *J. communis* var. *hemisphaerica* mainly in limestone (Pérez-Latorre and Cabezudo, 2011). There are numerous herbarium sheets from both taxa in the area, mainly in the herbaria of Cordoba University (COA), Granada University (GDA) and the Royal Botanic Garden of Madrid (MA). Some of these sheets were initially identified as *J. communis* var. *saxatilis*, and subsequently attributed to *J. communis* var. *hemisphaerica*. However, in two cases (MA153763, MA589195) the specimens show intermediate characters between these two taxa, apparently belonging to the hybrid described here, although genetic analysis would be necessary to confirm this. The only specimens with certainty of their hybrid character, because they have been subjected to genetic analysis, and deposited in a registered herbarium, are those collected by Adams in Sierra Nevada (BAYLU043718, BAYLU043721), which have therefore been selected for the typification of the hybrid.

Although *J. communis* nothosubsp. *guadarramica* Rivas Mart. was described as a hybrid between *J. communis* subsp. *nana* and *J. communis* subsp. *hemisphaerica*, their parents are actually *J. communis* var. *communis* and *J. communis* var. *saxatilis*, so it is an illegitimate name, synonym to *J. communis* nothovar. *intermedia*.

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Volatile compound profile of *Osmorhiza occidentalis* Torr. (Apiaceae) root essential oil

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ABSTRACT

Osmorhiza occidentalis Torr. is an essential-oil-bearing plant in the Apiaceae family. Previous research was conducted by the same group of researchers on the essential oil produced by and extracted from the above-ground plant parts (herb). The current study was conducted on the steam distilled essential oil from the roots of the same plant, *O. occidentalis*. The root essential oil was analyzed by gas chromatography (GC/FID, GC/MS) and was found to be comprised of 39 compounds. Prominent volatile compounds include sabinene (24.2%), o-cymene (2.3%), (Z)-β-ocimene (2.0%), γ-terpinene (19.6%), methyl chavicol (22.4%), 2-allyl-1,4-dimethoxybenzene (2.4%), and 1-allyl-2,4-dimethoxybenzene (18.4%). The current study establishes for the first time, to the best knowledge of the authors, the essential oil yield and composition of *O. occidentalis* root. These results provide fundamental data for substantiation of ethnobotanical applications and for future investigations on the secondary metabolites from this plant species. *Published online* ***www.phytologia.org*** *Phytologia 106(2): 33-38 (June 20, 2024). ISSN 030319430.*

KEY WORDS: Apiaceae, essential oil, gas chromatography, *Osmorhiza occidentalis*, root

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Osmorhiza occidentalis, commonly known as western sweet-cicely or mountain sweet-cicely, is a perennial herbaceous plant belonging to the Apiaceae family (WFO: accessed 12 April 2024). It is native to the western regions of North America, particularly the Intermountain Region extending into the Pacific Northwest of the United States and northward into Canada.

This aromatic plant is characterized by its finely divided, pinnate leaves and small yellow to greenish-white flowers arranged in umbels (Cronquist et al. 1997) (Fig. 1). The plant typically grows in moist, shaded areas such as forests, woodlands, and along stream banks. Pleasantly, one of the distinguishing features of *O. occidentalis* is its licorice-like aroma, which emanates from both its roots and foliage (Cronquist et al. 1997). This aroma is attributed to the presence of certain volatile compounds within the plant (Wilson et al. 2022).

Figure 1. Botanical illustration of *Osmorhiza occidentalis* whole plant (left) and close perspective of the root (right). The main stem (left) is truncated to focus on and detail the herbaceous and reproductive plant parts. Illustrated by Zach Nielsen.

Throughout history, indigenous peoples have utilized various parts of *O. occidentalis* for medicinal, culinary, and cultural purposes. The roots were often brewed into teas to treat respiratory issues such as colds and pneumonia (Murphy 1959), while the seeds and stems were used for flavoring foods and dyeing garments (Moerman 1998), respectively. Specifically, the Paiute and Shoshone peoples referred to the plant as "Bossowey" and valued its medicinal properties in treating various ailments (Murphy 1959; Uphof 1968).

While *O. occidentalis* has a rich ethnobotanical history, modern scientific research on its medicinal properties is relatively limited. However, some studies have identified compounds such as falcarindiol (Kern and Cardellina 1982) in the plant, which is known for its antifungal properties. The objective of this ongoing study was to extend the scope of prior research conducted by the same research team (Wilson et al. 2022). This study compares the root essential oil profile and yield with previous findings of the herb essential oil profile and yield.

MATERIALS AND METHODS

Osmorhiza occidentalis plant material was collected on July 17, 2023, from native populations located on public lands (Bureau of Land Management) on the Oquirrh Mountain Range in Tooele County, Utah, USA (40°28′15″ N 112°10′18″ W; 2737 m elevation). Both above-ground portions (during the flowering stage) and roots were collected to determine the weight, yield, and the composition of the volatile oil extracted from the herb and root. A representative voucher sample is held in the Young Living Aromatic Herbarium (YLAH): *O. occidentalis* Torr., Wilson 2023-01 (YLAH).

Organic chervil, *Anthriscus cerefolium* Hoffm. (Apiaceae), seeds were purchased (Johnny's Selected Seeds, Winslow, ME, USA), grown at the Young Living Research Greenhouse for a 3-month period, and the herb was harvested as inflorescence developed. The distilled essential oil was used as a reference standard for the identification of two compounds, 2-allyl-1,4-dimethoxybenzene and 1-allyl-2,4-dimethoxybenzene.

Plant material was prepared for laboratory-scale distillation as follows: the below-ground portion (root) was cleaned and dried. The above-ground portions (herb) and root were grouped separately, bagged, and stored at −20 ± 2 °C until steam distilled.

Laboratory-scale distillation was as follows: 1.5 L of water was added to 2-L steam generator that fed into a 2-L distillation chamber, plant material was accurately weighed and added to the distillation chamber, distillation for 1.5 h from pass-over by indirect steam, essential oil separated by a cooled condenser and Florentine flask. Essential oil samples were each filtered and stored at room temperature in a sealed amber glass bottle until analysis.

The percent yield was calculated as the ratio of the mass of processed plant material immediately before distillation to the mass of essential oil produced, multiplied by 100.

Essential oil samples were analyzed, and volatile compounds identified, by GC/MS using an Agilent 7890B GC/5977B MSD (Agilent Technologies, Santa Clara, CA, USA) and Agilent J&W DB-5, 0.25 mm × 60 m, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.1 µL of sample (20% soln. for essential oils in methylene chloride), 100:1 split ratio, initial oven temp. of 40 °C with an initial hold time of 5 min., oven ramp rate of 4.5 °C per min. to 310 °C with a hold time of 5 min. The electron ionization energy was 70 eV, scan range 35–650 amu, scan rate 2.4 scans per sec., source temp. 230 °C, and quadrupole temp. 150 °C. Volatile compounds were identified using the Adams volatile oil library (Adams 1997) using Chemstation library search in conjunction with retention indices. Note that limonene/β-phellandrene eluted as a single peak. Their amounts were determined by the ratio of masses 68 and 79 (limonene), 77 and 93 (β-phellandrene). Volatile compounds were quantified and are reported as a relative area percent by GC/FID using an Agilent 7890B GC and Agilent J&W DB-5, 0.25 mm × 60 m, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.1 µL of sample (20% soln. for essential oils in methylene chloride, 1% for reference compounds in ethanol, 0.1% soln. for C7–C30 alkanes in hexane), 25:1 split ratio, initial oven temp. of 40 °C with an initial hold time of 2 min., oven ramp rate of 3.0 °C per min. to 250 °C with a hold time of 3 min. Essential oil samples were analyzed in

triplicate by GC/FID to ensure repeatability of relative area % values. Compounds were identified using retention indices coupled with retention time data of reference compounds (MilliporeSigma, Sigma-Aldrich, St. Louis, MS, USA).

RESULTS AND DISCUSSION

Previously published research on the essential oil profile of *Osmorhiza occidentalis* herb by Wilson and associates (2022) was conducted on plant material from the same mountain range (Oquirrh Mountains). However, for both studies (Wilson et al. 2022; current), plant material was collected from two different populations of *O. occidentalis*, which lie approximately 250 meters apart and are separated by a dense Douglas fir (*Pseudotsuga menziesii*) forest. In the Oquirrh Mountain range, *O. occidentalis* appears to grow best at high altitudes (> 2500 m) and with either full sun exposure or partial shade, but it does not grow well under thick forest canopy.

Since plant material was collected from a different population of *O. occidentalis*, above-ground plant parts (herb) were once again collected and distilled for comparative analysis. Additionally, roots were collected and distilled to establish essential oil yield (Table 1) and essential oil composition (Table 2).

Table 1. Yield data, including mass of the plant material distilled (g), essential oil yield (g), and calculated yield (%) from *Osmorhiza occidentalis* herb and root samples.

Sample	Plant Mass Distilled (g)	EO Yield (g)	EO Yield (%)
Herb	410.08	0.77	0.19
Root	423.90	9.08	2.14

The calculated essential oil yield (*w/w*) of the herb in the current study (0.19%) was similar to the average calculated yield of herb from the previous study (0.12%) (Wilson et al. 2022). The calculated essential oil yield (*w/w*) of the root was 2.14%, or just over 11x the yield of the herb.

Table 2. Essential oil profile of *Osmorhiza occidentalis* herb and root samples. Reported values represent averages from samples analyzed in triplicate, which was done to ensure repeatability of values (standard deviation ≤ 0.7 for all compounds). Values less than 0.1% are denoted as trace (tr) and those not detected as nd. KI is the Kovat's Index value and was previously calculated by Robert Adams using a linear calculation on a DB-5 column (Adams 1997). Relative area percent was determined by GC/FID.

Compound Name	**KI**	**Herb EO (area %)**	**Root EO (area%)**
α-thujene	924	0.1	0.3
α-pinene	932	0.2	1.1
camphene	946	tr	tr
sabinene	969	9.8	24.2
β-pinene	974	0.1	0.6
myrcene	988	0.4	0.7
α-phellandrene	1002	0.1	tr
o-cresol methyl ether	1005	nd	tr
δ-3-carene	1008	nd	tr
α-terpinene	1014	tr	0.3
o-cymene	1022	2.1	2.3
limonene	1024	0.1	tr
β-phellandrene	1025	0.1	0.3
(*Z*)-β-ocimene	1032	6.1	2.0
(*E*)-β-ocimene	1044	0.2	0.1
γ-terpinene	1054	1.8	19.6

Compound Name	KI	Herb EO (area %)	Root EO (area%)
(*Z*)-sabinene hydrate	1065	tr	0.1
terpinolene	1086	0.3	0.1
3-isopropyl-2-methoxypyrazine	1090	nd	tr
linalool	1095	0.1	nd
(*E*)-sabinene hydrate	1098	nd	0.1
(*Z*)-4-methoxy thujane	1118*	nd	0.3
allo-ocimene	1128	0.6	0.2
citronellal	1148	tr	nd
terpinen-4-ol	1174	0.3	0.3
α-terpineol	1186	tr	0.1
methyl chavicol	1195	74.7	22.4
citronellol	1223	0.2	nd
thymol methyl ether	1232	nd	tr
pulegone	1233	tr	nd
neral	1235	0.1	nd
(*E*)-anethol	1282	1.3	0.2
bornyl acetate	1284	nd	tr
α-copaene	1374	nd	tr
2-allyl-1,4-dimethoxybenzene	1393*	nd	2.4
methyleugenol	1403	tr	1.6
1-allyl-2,4-dimethoxybenzene	1407*	tr	18.4
isoamyl benzoate	1433	nd	tr
bicyclogermacrene	1500	tr	0.6
kessane	1529	nd	tr
(*E*)-nerolidol	1561	tr	nd
(*E*)-p-methoxycinnamaldehyde	1562	tr	nd
spathulenol	1577	nd	tr
γ-eudesmol	1630	nd	tr
shyobunol	1688	nd	tr
benzyl benzoate	1759	tr	0.3
	sum	**98.6**	**98.5**

*KI not included in the Adam's library (1997). KI was manually calculated using alkane standards on a DB-5 column.

As with previous findings (Wilson et al. 2022), the composition of the herb essential oil is largely composed of five volatile compounds, including sabinene (9.8%), o-cymene (2.1%), (*Z*)-β-ocimene (6.1%), γ-terpinene (1.8%), and methyl chavicol (74.7%), which, together, comprise approximately 95% of the identified compounds. The same five volatile compounds comprise approximately 70% of the root sample (sabinene 24.2%, o-cymene 2.3%, (*Z*)-β-ocimene 2.0%, γ-terpinene 19.6%, methyl chavicol 22.4%). Additionally, 2-allyl-1,4-dimethoxybenzene and 1-allyl-2,4-dimethoxybenzene are other prominent compounds of the root, comprising 2.4% and 18.4% of the essential oil profile, respectively.

These same two compounds, 2-allyl-1,4-dimethoxybenzene and 1-allyl-2,4-dimethoxybenzene, are not in the Adams' library (1997). Their preliminary identification was made by referencing the 2020 NIST mass spectral library and referencing previously published research on chervil herb (*Anthriscus cerefolium*), which contains both compounds (Baser et al. 1998; Chizzola 2011; Lemberkovics et al. 1994). Their identification was confirmed by distilling and analyzing in-house cultivated chervil herb, which contained both compounds with matching mass spectra and compound elution times (Table 3). Additionally, chervil herb and *O. occidentalis* root samples were analyzed using GC columns with different stationary phases (Rtx-1, DB-5) to confirm that elution times of the two compounds still matched between samples. Prominent ions for 2-allyl-1,4-dimethoxybenzene and 1-allyl-2,4-dimethoxybenzene include 103, 135, 178 and 121, 147, 178, respectively.

Table 3. Essential oil profile of *Anthriscus cerefolium* (chervil) herb and *Osmorhiza occidentalis* root samples. Reported values represent averages from samples analyzed in triplicate, which was done to ensure repeatability of values (standard deviation ≤ 1.0 for all compounds). KI is the Kovat's Index value and was previously calculated by Robert Adams using a linear calculation on a DB-5 column (Adams 1997). Relative area percent was determined by GC/FID.

Compound Name	KI	Chervil Herb EO (area %)	Root EO (area%)
β-pinene	974	2.2	0.6
methyl chavicol	1195	20.4	22.4
2-allyl-1,4-dimethoxybenzene	1393*	3.2	2.4
1-allyl-2,4-dimethoxybenzene	1407*	68.3	18.4

*KI not included in the Adam's library (1997). KI was manually calculated using alkane standards on a DB-5 column.

CONCLUSIONS

Findings from the current study confirm those from previous research on the essential oil from the above-ground portions (herb) of *Osmorhiza occidentalis*. Namely, that the herb essential oil profile is largely composed of five volatile compounds (sabinene, o-cymene, (*Z*)-β-ocimene, γ-terpinene, methyl chavicol) and that volatile compound profile variability exists from sample-to-sample (Wilson et al. 2022).

Unfortunately, the limited population size of *O. occidentalis* on the Oquirrh Mountain range, particularly on public lands, did not allow for collection and distillation of additional root essential oil samples. An investigation of additional samples in the future would provide an understanding if, similar to herb samples, yield and volatile compound profile variability exist from sample-to-sample and/or population-to-population in root samples.

In the current study, it was found that the root essential oil of *O. occidentalis* had a much higher yield (> 11x) compared to the herb of the same plant. Also, while the volatile compound profile of the root essential oil does somewhat overlap with that of the herb, the root essential oil contains two prominent compounds, 2-allyl-1,4-dimethoxybenzene and 1-allyl-2,4-dimethoxybenzene, that were not previously detected in the herb. Future research could investigate reasons for the differences in essential oil yield and volatile compound profiles from the root and herb of *O. occidentalis*.

The current study focuses on the identification and quantification of volatile compounds from *O. occidentalis*. Future research could use other analytical techniques (LC/MS) to investigate concentrations of falcarindiol and other non-volatile compounds. Additionally, future studies could investigate the application of both volatile and non-volatile secondary metabolites from *O. occidentalis*.

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